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VARIABILITY IN CRASSULACEAN ACID METABOLISM: A SURVEY OF NORTH CAROLINA SUCCULENT SPECIES

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The correlation between succulence and Crassulacean acid metabolism (CAM) was investigated in 28 succulent species growing in various habitats throughout North Carolina. Three species (*Opuntia compressa*, *Agave virginica*, and *Tillandsia usneoides*) exhibited diurnal fluctuations in tissue titratable acidity, nighttime uptake of $^{14}CO_2$, and a high carbon isotope ratio ($\delta^{13}C$), all indicators of CAM. Seven species displayed one or two characteristics of CAM in situ yet yielded lower $\delta^{13}C$ values, indicating a partial or total restriction of atmospheric CO_2 uptake to the C_3 photosynthetic system: *Yucca gloriosa*, *Sesuvium maritimum*, *Talinum teretifolium*, *Diamorpha smallii*, *Sedum pusillum*, *Sedum nevii*, and *Sedum telephioides*. Several of these species were apparently capable of utilizing the CAM pathway to fix internal respiratory CO_2. The results emphasize that one photosynthetic pathway does not characterize all succulents in North Carolina.

Introduction

The relationship between succulence and Crassulacean acid metabolism (CAM) was questioned by KLUGE and TING (1978). With the discovery of CAM in nonsucculent epiphytic bromeliads, such as *Tillandsia usneoides* (COUTINHO 1969), it was clear that at least some epiphytic CAM plants might not be succulent. It is also possible that, in at least some plant families, there exist terrestrial species capable of CAM which do not have succulent photosynthetic organs (RAO, SWAMY, and DAS 1979; TEERI, TONSOR, and TURNER 1981).

To examine the relationship between CAM and succulence, this study was designed to test for the presence of CAM in as many succulents as possible, using plants growing in various habitats in North Carolina. Few species of CAM plants have been reported from the mesic southeastern United States, the most extensively studied species being *T. usneoides* (MARTIN, CHRISTENSEN, and STRAIN 1981; MARTIN and SIEDOW 1981; MARTIN 1982). The only other plants in the southeastern United States reported to be CAM are *Opuntia humifusa* (BENDER [1971]; probably the same species as *O. compressa* in this study), *Sedum telephioides* (MATHUR, NATARELLA, and VINES 1978), and *Yucca filamentosa* (SZAREK and TING [1977]; but see Results and discussion below).

Material and methods

The criteria for including a species as a succulent were broadly defined to include a maximum number of species. Any species described by RADFORD, AHLES, and BELL (1968) as having succulent, fleshy, or thick leaves or photosynthetic stems was included in the study. In addition, several nonsucculent species of genera previously reported to have CAM were sampled, e.g., *Yucca* and *Tillandsia*. Only species growing in the state of North Carolina (one study site was located in South Carolina near the North Carolina border) were included in the study (species, habitat types, sites, and sampling dates in table 1).

For calculations of $^{14}CO_2$ uptake rates, photosynthetic organs—either detached shoots, leaves, or portions of leaves (stems in *Opuntia compressa*)—were placed in a Plexiglas chamber (70 ml vol) and exposed to $^{14}CO_2$ (22 μCi·liter^{-1}) in air (315 μl·liter^{-1} CO_2) for 1 min at a flow rate of 500 ml·min^{-1}. Plants (three replicates per species) were sampled once during midday and once near midnight. After exposure, plant parts were immediately placed in vials at dry-ice temperature (−78.5 C) and subsequently oven-dried (65 C), cooled in a desiccator, and weighed. They were oxidized with perchloric acid and H_2O_2 at 45 C for 12 h. A scintillation cocktail of ethylene glycol monoethyl ether and toluene-PPO (2,5-diphenyloxazole) was added after cooling, and the samples were counted in a Beckman LS-250 liquid scintillation counter. Further details of the $^{14}CO_2$-exposure, oxidation, and counting techniques are presented in MARTIN et al. (1981).

For subsequent determination of tissue titratable acidity, leaves or whole plants (five replicates per species) were collected, placed in plastic bags, and immediately frozen on dry ice. In the laboratory, the plant material was ground in distilled water with a mortar and pestle, vacuum filtered, and titrated to pH 7.0 with 0.01 N NaOH. The filtered material was dried at 65 C, cooled in a desiccator, and weighed. Further details of this technique are described in MARTIN et al. (1981).

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Simultaneous with sampling for $^{14}CO_2$ uptake rates and titratable acidity fluctuations, samples of mature photosynthetic tissue were collected and dried (65 C) for mass spectrometric determinations of $\delta^{13}C$ values, expressed relative to the PDB standard (TEERI 1981). The $\delta^{13}C$ values for each species (tables 2–6) are means of two determinations; the difference between the replicates was always less than 1.9‰.

Voucher specimens were collected and deposited in the Duke University Herbarium.

Results and discussion

None of the salt marsh succulent species exhibited nighttime $^{14}CO_2$ fixation (table 2). A significant nocturnal increase in tissue titratable acidity was found only in *Sesuvium maritimum* and may indicate nighttime fixation of respiratory CO_2. Fluctuations in tissue acidity without accompanying atmospheric CO_2 fixation were also measured in *Opuntia basilaris* (SZAREK and TING 1974) and several other succulent species (HANSCOM and TING 1978).

The $\delta^{13}C$ value integrates the lifetime photosynthetic history of a plant or plant part. Thus, the tissue from a C_4 plant will yield a carbon isotope ratio of ca. −13‰, that of a C_3 plant about −27‰, while that from a CAM plant can vary between these values, depending on what proportion of time the C_3 and C_4 carboxylating enzymes were functioning in the uptake of atmospheric CO_2 throughout the life of the plant (BLACK 1976).

The seven salt marsh species yielded $\delta^{13}C$ values characteristic of C_3 plants (table 2). Based on these data, it appears highly unlikely that any of the salt marsh succulents examined here take up atmospheric CO_2 via the CAM pathway. Several of these species were examined previously. *Atriplex patula* is a C_3 species (BJÖRKMAN 1973). Although dark $^{14}CO_2$ uptake was reported in *Salicornia europaea*, *S. virginica*, and *Borrichia frutescens* (WEBB and BURLEY 1965),

TABLE 1

SPECIES, STUDY SITES, AND SAMPLING DATES FOR THE SUCCULENT PLANTS EXAMINED

Habitat and species	Date sampled (1979)	Location
Salt marsh:		
Salicornia virginica	May 21–22	Holden Beach, N.C. (Brunswick County)
Borrichia frutescens	May 21–22	Holden Beach, N.C.
S. europaea	August 17–18	State Highway 58, N.C. (Carteret County)
Aster subulatus	August 17–18	Oak Island, N.C. (Brunswick County)
Atriplex patula	August 17–18	Oak Island, N.C.
Suaeda linearis	August 17–18	Oak Island, N.C.
Sesuvium maritimum	August 17–18	Oak Island, N.C.
Beach sand:		
Iva imbricata	May 21–22	Holden Beach, N.C.
Cakile edentula	May 21–22	Holden Beach, N.C.
Amaranthus pumilus	May 21–22	Holden Beach, N.C.
Yucca gloriosa	May 21–22	Holden Beach, N.C.
Ipomoea stolonifera	August 17–18	Oak Island, N.C.
Salsola kali	August 17–18	Oak Island, N.C.
Granite outcrop:		
Arenaria uniflora	April 22–23	40-Acre Rock, S.C. (Lancaster County)
Arenaria groenlandica	April 22–23	40-Acre Rock, S.C.
Yucca filamentosa	April 22–23	40-Acre Rock, S.C.
Talinum teretifolium	April 22–23	40-Acre Rock, S.C.
Sedum pusillum	April 22–23	40-Acre Rock, S.C.
Diamorpha smallii	April 22–23	40-Acre Rock, S.C.
Rock ledge:		
Sedum nevii	August 6–7	Blue Ridge Parkway, N.C. (Buncombe County)
Sedum telephioides	August 6–7	Blue Ridge Parkway, N.C.
Talinum teretifolium	August 6–7	Blue Ridge Parkway, N.C.
Portulaca smallii	August 31–September 1	State Highway 49, N.C. (Cabarrus County)
Opuntia compressa	August 31–September 1	State Highway 49, N.C.
Agave virginica	August 31–September 1	Denton, N.C. (Randolph County)
Weedy:		
Portulaca pilosa	September 15–16	Wadesboro, N.C. (Anson County)
P. oleracea	September 9–10	Durham, N.C. (Durham County)
Stream bank:		
Sedum ternatum	September 9–10	Duke Forest, N.C. (Orange County)
Epiphytic:		
Tillandsia usneoides	May 13–14	Bladen Lakes State Forest, N.C. (Bladen County)

this study and those of WELKIE and CALDWELL (1970), ANTLFINGER and DUNN (1979), and KURAMOTO and BREST (1979) indicate that these are C_3 species. While many species of *Suaeda* are reportedly C_4 plants (DOWNTON 1975), *S. linearis* lacks Kranz anatomy (WELKIE and CALDWELL 1970), and the $\delta^{13}C$ value measured for this species in this study (−25‰; table 2) indicates the C_3 photosynthetic pathway. This species, therefore, also appears to be C_3.

Of the seven beach sand species examined, only *Yucca gloriosa* exhibited significantly higher levels of tissue acidity at sunrise than at sunset (table 3). This occurred without a concomitant nocturnal uptake of $^{14}CO_2$, again suggesting a nocturnal fixation of respiratory CO_2, as in *S. maritimum*. The carbon isotope ratio (−22‰) of *Y. gloriosa* is intermediate between typical C_3 and C_4 values and may indicate an ability to assimilate atmospheric CO_2 both at night and during the day. CAM has been found in other *Yucca* species (MOONEY, TROUGHTON, and BERRY 1974; COCKBURN, TING, and STERNBERG 1979) though not in all, e.g., *Y. filamentosa* (BENDER 1971; this study).

The lack of a significant difference in tissue acidity at sunrise and sunset and the absence of substantial nocturnal $^{14}CO_2$ uptake indicate that the remaining beach sand species were not utilizing the CAM pathway when sampled (table 3). The high $\delta^{13}C$ value of *Salsola kali* (−11‰) is typical of a C_4 species, and previous studies have confirmed this species to be C_4 (TREGUNNA and DOWNTON 1967; WELKIE and CALDWELL 1970). All *Amaranthus* species examined thus far are C_4 (WELKIE and CALDWELL 1970; DOWNTON 1975), and *A. pumilus* appears to be no exception. *Iva imbricata*, *Cakile edentula*, and *Ipomoea stolonifera* exhibit characteristics typical of C_3 plants; there are no known reports of C_4 or CAM in these three genera. Low rates of nighttime $^{14}CO_2$ uptake were found in *A. pumilus* and *C. edentula* and were significantly different from zero (95% confidence intervals did not include zero). Further work is required before these nocturnal CO_2 uptake rates can be attributed

TABLE 2

TISSUE TITRATABLE ACIDITIES, $^{14}CO_2$ UPTAKE RATES, AND $\delta^{13}C$ VALUES FOR SALT MARSH SUCCULENT SPECIES

SPECIES	MEAN ± SD TITRATABLE ACIDITY (μeq·g DW^{-1})			MEAN ± SD $^{14}CO_2$ UPTAKE RATE (mg CO_2·h^{-1}·g DW^{-1})			
	Sunrise	Sunset	Significance[a]	Night	Day	Significance[b]	$\delta^{13}C$ (‰)
Salicornia virginica	36 ± 7	81 ± 40	NS	.0 ± .0	16.1 ± 5.9	***	−26
S. europaea	65 ± 35	49 ± 15	NS	.0 ± .0	35.4 ± 4.0	**	−27
Borrichia frutescens	37 ± 15	34 ± 12	NS	.0 ± .0	26.5 ± 5.6	*	−27
Aster subulatus	42 ± 16	33 ± 8	NS	.0 ± .0	6.0 ± 4.0	NS	−26
Atriplex patula	52 ± 15	39 ± 21	NS	.0 ± .0	6.3 ± .7	**	−25
Suaeda linearis	94 ± 22	79 ± 39	NS	.0 ± .0	16.2 ± 4.7	*	−25
Sesuvium maritimum	50 ± 4	21 ± 13	**	.0 ± .0	8.8 ± 2.2	*	−26

[a] Levels of significance indicate results of *t*-test (or modified *t'*-test in cases of heteroscedasticity; SOKAL and ROHLF [1969]) of the differences between mean sunrise acidity and mean sunset acidity. NS = not significant.* = $P < .05$, ** = $P < .01$, *** = $P < .001$. No. = 5.

[b] Levels of significance for same tests of the differences between night and day $^{14}CO_2$ uptake rates. No. = 3.

TABLE 3

TISSUE TITRATABLE ACIDITIES, $^{14}CO_2$ UPTAKE RATES, AND $\delta^{13}C$ VALUES FOR BEACH SAND SUCCULENT SPECIES

SPECIES	MEAN ± SD TITRATABLE ACIDITY (μeq·g DW^{-1})			MEAN ± SD $^{14}CO_2$ UPTAKE RATE (mg CO_2·h^{-1}·g DW^{-1})			
	Sunrise	Sunset	Significance	Night	Day	Significance	$\delta^{13}C$ (‰)
Iva imbricata	55 ± 29	31 ± 12	NS	.0 ± .0	62.0 ± 4.7	**	−27
Cakile edentula	80 ± 34	115 ± 38	NS	.1 ± .0	55.5 ± 21.4	*	−29
Ipomoea stolonifera	56 ± 9	49 ± 17	NS	.0 ± .0	7.8 ± 5.9	NS	−27
Amaranthus pumilus	136 ± 93	111 ± 51	NS	.2 ± .1	20.8 ± 2.9	**	−13
Salsola kali	20 ± 14	56 ± 38	NS	.0 ± .0	3.5 ± 2.6	NS	−11
Yucca gloriosa	331 ± 138	123 ± 24	*	.0 ± .0	6.2 ± 2.0	*	−22

NOTE.—Levels of significance determined as in table 2.

to enzymatic fixation or to contamination of the cut leaf surfaces.

Among the six succulent species found on granite outcrops, the two *Arenaria* species and *Y. filamentosa* exhibited no nocturnal uptake of $^{14}CO_2$, no difference in titratable acidity at sunrise or sunset, and low, C_3-like $\delta^{13}C$ values (−26‰ and −28‰; table 4). It appears that these species were functioning as C_3 plants. *Talinum teretifolium* exhibited rates of nighttime $^{14}CO_2$ uptake not significantly different from zero and much lower than daytime rates; yet the tissue acidity at sunrise was significantly higher than that at sunset (table 4). Conversely, *Sedum pusillum* exhibited a low but significant (relative to zero) rate of nocturnal $^{14}CO_2$ uptake, but no significant fluctuation in tissue acid content. Though CAM has been reported in many species of *Sedum* (SZAREK and TING 1977), *S. pusillum* may have some ability to utilize the CAM photosynthetic pathway, but its carbon isotope ratio (−26‰) indicates that only the C_3 pathway functions in the fixation of atmospheric CO_2. A similar C_3-like $\delta^{13}C$ value was found in *T. teretifolium* in spite of the nocturnal accumulation of acid. These results indicate the possible utilization of CAM for limited nighttime uptake of atmospheric CO_2 in *S. pusillum* and for nighttime uptake of internal, respiratory CO_2 in *T. teretifolium*. Fluctuations in titratable acidity in *T. guadalupense* were reported by KLUGE and TING (1978).

Diamorpha smallii displayed nighttime $^{14}CO_2$ uptake rates that were low but significantly higher than daytime rates, and tissue acidity at sunrise was significantly higher than that at sunset (table 4). In spite of these indications of in situ CAM, the carbon isotope ratio (−28‰) suggests that the majority of carbon fixed during the life of the plant was via the C_3 carboxylation pathway. *Diamorpha smallii* seedlings grow in vernal pools on granite outcrops during the mesic spring (MCVAUGH 1943). These pools evaporate by late spring or early summer, exposing the plant to potentially great water stress in the shallow, sandy soil. During this time, switching from the C_3 to the CAM pathway could potentially result in the conservation of water while the plants remained metabolically active (KLUGE and TING 1978). A similar switch in photosynthetic pathways upon increasing water stress also was observed in annual halophytic *Mesembryanthemum* species (WINTER et al. 1978; WINTER and TROUGHTON 1978).

Several succulent species that grow on rock ledges throughout North Carolina exhibited a variety of ecophysiological traits (table 5). As are numerous other *Portulaca* species (DOWNTON 1975), *P. smallii* appears to be a C_4 plant. There was no nighttime $^{14}CO_2$ uptake, no difference in tissue acidity at sunrise relative to sunset, and a high $\delta^{13}C$ value (−13‰). There were several indications of CAM in *Opuntia compressa:* higher, though not significantly so, rates of nocturnal $^{14}CO_2$ uptake relative to the daytime rate (nighttime rates were significantly different from zero; daytime rates were not), significantly higher tissue acidity at sunrise than at sunset, and a high $\delta^{13}C$ value characteristic of an obligate CAM plant (−12‰). Several other studies support these findings of CAM in *O. compressa* (CONDE and KRAMER [1975]; KOCH and KENNEDY [1980*b*], who reported *O. humifusa* as a CAM plant; this species is synonymous with *O. compressa* according to RADFORD et al. [1968]).

Although many desert *Agave* species are CAM plants (SZAREK and TING 1977), no reports of the photosynthetic pathway in *A. virginica* could be found. This may be attributable to the relative scarcity of the plant, at least in North Carolina. A large, significant difference between tissue acid content at sunrise and sunset, as well as low levels of nighttime $^{14}CO_2$ uptake (significantly different from zero), indicates CAM in this species (table 5). The $\delta^{13}C$ value (−16‰) also supports these indications of CAM in *A. virginica*.

Although *Sedum nevii* exhibited no nocturnal $^{14}CO_2$ uptake and yielded a C_3-like $\delta^{13}C$ value

TABLE 4

TISSUE TITRATABLE ACIDITIES, $^{14}CO_2$ UPTAKE RATES, AND $\delta^{13}C$ VALUES FOR GRANITE OUTCROP SUCCULENT SPECIES

SPECIES	MEAN ± SD TITRATABLE ACIDITY (μeq·g DW^{-1})			MEAN ± SD $^{14}CO_2$ UPTAKE RATE (mg CO_2·h^{-1}·g DW^{-1})			$\delta^{13}C$ (‰)
	Sunrise	Sunset	Significance	Night	Day	Significance	
Arenaria uniflora	108 ± 21	129 ± 39	NS	.0 ± .0	3.3 ± 1.4	NS	−28
A. groenlandica	97 ± 26	120 ± 21	NS	.0 ± .0	4.4 ± .8	*	−28
Yucca filamentosa	85 ± 36	70 ± 38	NS	.0 ± .0	2.9 ± 1.4	NS	−26
Talinum terelifolium	798 ± 173	280 ± 65	***	.1 ± .1	.8 ± .8	NS	−27
Sedum pusillum	211 ± 49	177 ± 20	NS	.1 ± .0	.9 ± .7	NS	−26
Diamorpha smallii	454 ± 45	305 ± 52	**	.2 ± .0	.0 ± .0	**	−28

NOTE.—Levels of significance determined as in table 2.

(−27‰), tissue titratable acidity at sunrise was significantly higher than at sunset (table 5). As with several species above, these findings may indicate refixation of nocturnal respiratory CO_2. *Sedum telephioides*, reported as a CAM plant (SZAREK and TING 1977), exhibited characteristics typical of CAM plants: a significantly greater rate of nocturnal $^{14}CO_2$ uptake relative to daytime (and significantly greater than zero), and a higher tissue acid content at sunrise than at sunset. On the other hand, its intermediate $\delta^{13}C$ value (−23‰) suggests the importance of the C_3 pathway to this apparently facultative CAM plant.

In addition to the populations at the granite outcrop site, *T. teretifolium* was also found on rock ledges in the mountains of North Carolina. Similar to the granite outcrop plants, the rock ledge *T. teretifolium* exhibited significantly greater tissue acidity at sunrise than at sunset (table 5). However, zero nocturnal and daytime $^{14}CO_2$ uptake rates were found in the rock ledge plants. The nighttime increase in tissue acidity may be attributed to refixation of respiratory CO_2. Similar C_3-like carbon isotope ratios were found in both populations of *T. teretifolium*, emphasizing the predominance of the C_3 pathway for fixing atmospheric CO_2 in this species, in spite of the ability to utilize the CAM pathway.

The four remaining succulent species examined for the presence of CAM in this study were found in several different habitats: *P. pilosa* in a sandy, disturbed area, *P. oleracea* in urban sidewalk crevices, *Sedum ternatum* on a stream bank in an oak-hickory forest, and *Tillandsia usneoides*, epiphytic on an oak tree. Both *Portulaca* species have been reported as C_4 species (DOWNTON 1975), and the ability to utilize the CAM pathway was reported for *P. oleracea* (KOCH and KENNEDY 1980*a*). In this study, neither species exhibited nighttime $^{14}CO_2$ uptake, no differences between tissue acid content at sunrise and sunset were found, and the $\delta^{13}C$ values were C_4-like (−11‰, −13‰; table 6). *Sedum ternatum* exhibited characteristics typical of C_3 species: no nighttime $^{14}CO_2$ uptake, no differences in tissue acid content at sunrise and sunset, and a C_3-like $\delta^{13}C$ value (−30‰; table 6). Previous work with *T. usneoides* in North Carolina established the predominance of the CAM pathway in this epiphyte

TABLE 5

TISSUE TITRATABLE ACIDITIES, $^{14}CO_2$ UPTAKE RATES, AND $\delta^{13}C$ VALUES FOR ROCK LEDGE SUCCULENT SPECIES

SPECIES	MEAN ± SD TITRATABLE ACIDITY (μeq·g DW^{-1}) Sunrise	Sunset	Significance	MEAN ± SD $^{14}CO_2$ UPTAKE RATE (mg CO_2·h^{-1}·g DW^{-1}) Night	Day	Significance	$\delta^{13}C$ (‰)
Portulaca smallii	1,435 ± 542	1,291 ± 165	NS	.0 ± .0	8.3 ± 2.1	***	−13
Opuntia compressa	1,002 ± 598	173 ± 69	*	.3 ± .1	.1 ± .1	NS	−12
Agave virginica	6,238 ± 1,333	167 ± 74	***	.1 ± .0	.2 ± .1	NS	−16
Sedum nevii	172 ± 39	65 ± 46	**	.0 ± .0	.4 ± .4	NS	−27
Sedum telephioides	1,003 ± 210	121 ± 43	***	.5 ± .2	.0 ± .0	*	−23
Talinum teretifolium	607 ± 213	203 ± 57	*	.0 ± .0	.0 ± .0	NS	−26

NOTE.—Levels of significance determined as in table 2.

TABLE 6

TISSUE TITRATABLE ACIDITIES, $^{14}CO_2$ UPTAKE RATES, AND $\delta^{13}C$ VALUES FOR WEEDY, STREAMBANK, AND EPIPHYTIC SUCCULENT SPECIES

SPECIES	MEAN ± SD TITRATABLE ACIDITY (μeq·g DW^{-1}) Sunrise	Sunset	Significance	MEAN ± SD $^{14}CO_2$ UPTAKE RATE (mg CO_2·h^{-1}·g DW^{-1}) Night	Day	Significance	$\delta^{13}C$ (‰)
Weedy:							
Portulaca pilosa	258 ± 65	219 ± 60	NS	.0 ± .0	5.5 ± 2.8	NS	−11
P. oleracea	147 ± 63	186 ± 116	NS	.0 ± .0	6.1 ± 1.7	*	−13
Streambank:							
Sedum ternatum	74 ± 28	60 ± 26	NS	.0 ± .0	1.9 ± .1	***	−30
Epiphytic:							
Tillandsia usneoides	500 ± 137	119 ± 37	*	.4 ± .2	.0 ± .0	***	−15

NOTE.—Levels of significance determined as in table 2.

(MARTIN et al. 1981; MARTIN and SIEDOW 1981). The lack of daytime $^{14}CO_2$ uptake, significantly higher tissue acid content at sunrise than at sunset, and the high $\delta^{13}C$ value characteristic of the C_4 carboxylating enzyme system (−15‰; table 6) further support the previous findings.

Several methodological problems were unavoidable in a study of this nature. To include as many succulent species as possible, sample sizes were small, often resulting in large but nonsignificant differences between means; environmental conditions were not consistent between sample dates, though most species were sampled after several days of drought; and measurements were made at one point in time only. Therefore, this study cannot preclude the possibility that a species may exhibit CAM behavior at times other than that sampled.

The results of this study emphasize the lack of correlation between CAM and succulence; i.e., the presence of succulence in a plant is not necessarily a good indication of the presence of a functioning CAM pathway. On the other hand, except for *T. usneoides* and possibly *Y. gloriosa*, all of the CAM and facultative CAM species investigated in this study were succulent.

This study also emphasizes the potential difficulty in attempting to classify species with regard to their photosynthetic pathway. Several species in this study exhibited in situ characteristics of one type of metabolism, yet their carbon isotope discrimination ratio indicated the predominance of another pathway in the initial uptake of atmospheric CO_2 throughout the life of the plant.

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